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Apparently Normal Mitral Valves in Patients With Heart Failure Demonstrate Biochemical and Structural Derangements

An Extracellular Matrix and Echocardiographic Study

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OBJECTIVES This study assessed apparently normal mitral valves from patients with congestive heart failure (CHF) using biochemical and echocardiographic measures of extracellular matrix (ECM) and anatomy.

BACKGROUND Mitral regurgitation (MR) is frequently found in patients with CHF. This MR is considered purely functional, yet animal studies suggest that altered left ventricular (LV) function leads to increased cellularity and fibrosis of the mitral valve. Therefore, we hypothesized that patients with CHF might have partly organic MR, via dysfunctional valvular remodeling.

METHODS Mitral valves from transplant recipient hearts of patients with CHF (23 dilated, 14 ischemic) were analyzed for deoxyribonucleic acid (DNA), collagen, glycosaminoglycan (GAG), and water concentrations and compared with autopsy controls. Cardiac dimensions and functional parameters (measured from recent echocardiograms) were compared with biochemical parameters using a repeated measures generalized linear model.

RESULTS The mitral valves in CHF had up to 78% more DNA ($p < 0.03$), 59% more GAGs ($p < 0.02$), and 15% more collagen ($p < 0.007$), but 7% less water ($p < 0.05$) than normal. The absence of anterior leaflet redundancy was associated with these deranged biochemical measures ($p < 0.03$). Associations were found between leaflet thickness and DNA concentration (+, $p = 0.003$), annular diameter and chordal collagen (+, $p = 0.03$), and water concentration and both left atrial diameter (−, $p = 0.008$) and LV collagen concentration (−, $p = 0.04$).

CONCLUSIONS Mitral valves in CHF are biochemically different from normal, with ECM changes that are influenced by the altered cardiac dimensions. This remodeling suggests that MR in patients with CHF may not be purely functional, and that these valves are not "normal." (J Am Coll Cardiol 2005;45:54–61) © 2005 by the American College of Cardiology Foundation

Mitral regurgitation (MR) is present in more than half of all patients with ischemic or idiopathic dilated cardiomyopathy (DCM) resulting in congestive heart failure (CHF) (1,2).

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Because the mitral valve in these patients often appears normal on echocardiographic or gross pathologic examination, this MR is generally considered to be a functional consequence of alterations in the cardiac geometry. In functional MR, dilation of the ventricle and annulus or abnormal wall motion resulting from an ischemic event distorts the geometry of the mitral valve and alters its normal loading patterns, which then produces MR (3,4). Prolonged alteration to the normal valvular loading patterns, however, has been shown to up-regulate procollagen, collagen, and deoxyribonucleic acid (DNA) synthesis within mitral valve leaflets of animal models (5–7). Thus, it appears likely that MR may begin as purely functional but may be subsequently linked to microstructural changes or dysfunctional "remodeling" of the valve tissues. We hypothesize that mitral valves from patients with CHF will demonstrate this dysfunctional remodeling, and that the biochemical changes in the valvular extracellular matrix (ECM) are related to the cardiac distortion and dysfunction resulting from CHF. Therefore, the objectives of this study were first to quantify the ECM changes in mitral valve tissues from patients with CHF relative to normal control subjects, and then to examine the relationships between the matrix and cardiac and valvular dimensions and function.

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Abbreviations and Acronyms

CHF = congestive heart failure
DCM = dilated cardiomyopathy
DNA = deoxyribonucleic acid
ECM = extracellular matrix
GAG = glycosaminoglycan
ICM = ischemic cardiomyopathy
LV = left ventricular
MR = mitral regurgitation
TEE = transesophageal echocardiography
TTE = transthoracic echocardiography

METHODS

Subjects. The study subjects consisted of 37 patients with HF who received a heart transplant between September 1998 and August 2001 (Table 1). These patients were previously diagnosed with CHF on the basis of severe left ventricular (LV) systolic dysfunction (ejection fraction <25%) and either severe LV dilation or a history of atherosclerosis, coronary artery disease, or myocardial infarction. Ischemic cardiomyopathy (ICM) (n = 14) was distinguished from DCM (n = 23) on the basis of the presence of segmental wall motion abnormalities and the presence of significant obstructive coronary disease. Echocardiograms that adequately demonstrated the cardiac geometry (transthoracic) and the mitral valve (transesophageal) were available for many of these patients (Table 1). Patients who had mitral valve surgery before transplantation were excluded from the study. The research use of these patient tissues was approved by the Cleveland Clinic Foundation Institutional Review Board.

There were separate normal control groups for the different components of the study; in each component the control group matched the mean age and gender proportions of the patient group (Table 1). For the biochemical comparisons, both groups were predominantly men (CHF group 73% vs. control group 68%) and of similar age (CHF group 56 ± 14 years vs. controls group 50 ± 13 years). The biochemical control group consisted of normal control mitral valves obtained from autopsies of persons who died of causes other than cardiovascular/cardiac disease or sudden death; cadavers were stored at 4°C for <24 h before autopsy. Because valve tissues kept at 4°C retain their matrix-based mechanical properties for up to five days (8), we believe that the ECM in these cadaveric valves was not measurably degraded postmortem. Because echocardiograms were not available for these autopsy subjects, separate control groups were constructed for the anatomic and functional comparisons. The transthoracic echocardiogram (TTE) group consisted of patients 57 ± 12 years old (vs. 57 ± 8 years for control group) with 76% male patients (vs. 75%). Likewise, the transesophageal echocardiogram (TEE) group of patients had a mean age of 57 ± 7 years (vs. 57 ± 6 years for control group) with 91% male patients (vs. 86%). The TTE and TEE control groups (Table 1) were selected retroactively from a database of patients who underwent echocardiography between July 2002 and June 2003 and who did not demonstrate any cardiac or valvular disease.

Specimen preparation. The transplant recipient hearts were obtained immediately after transplantation and pathologic inspection. The mitral valve was dissected free and trimmed into leaflet and chordal specimens. The anterior leaflets were divided into the free edge (rough zone) and central region (clear zone). The posterior leaflet was not subdivided. The chordae were subdivided by insertion into either the posterior or anterior leaflet, in either the basal or marginal position. All the chordae from each group were pooled to obtain sufficient sample mass. In addition, a 100-mg section of the recipient heart left ventricle was reserved to measure LV collagen concentration.

Biochemical assays. The wet weights were determined, and water concentration was measured by lyophilizing the samples for 16 h and then weighing again when dry. To solubilize the tissues, each sample was minced, placed in 1 ml of 100 mM ammonium acetate (pH = 7), and incubated with 100 μl of proteinase-K (10 mg/ml) at 60°C for 16 h as previously described (9). The solubilized samples were then heated at 100°C for 10 min to inactivate the proteinase-K, and 100-μl aliquots were taken to measure the hydroxyproline (for collagen), DNA (for cellularity), and hexuronic acid concentration (for glycosaminoglycans [GAGs]) (9). The measured values from each sample were divided by the sample dry weights to obtain normalized concentrations.

Table 1. Subject Characteristics

Group	Subjects	Gender (F/M)	Age (yrs)	Diagnosis (DCM/ICM)	Total Number of Specimens Analyzed		
					Anterior Leaflet	Posterior Leaflet	Chordae
Biochemistry							
Controls	25	17/8	50 ± 13	—	44	23	74
Transplants	37	27/10	56 ± 14	23/14	47	34	79
Transthoracic echocardiography							
Controls	8	6/2	57 ± 8	—			
Transplants	33	25/8	57 ± 12	20/13			
Transesophageal echocardiography							
Controls	7	6/1	57 ± 6	—			
Transplants	11	10/1	57 ± 7	6/5			

DCM = dilated cardiomyopathy; ICM = ischemic cardiomyopathy.

Dry weight was chosen as the normalizing criteria to avoid possible confounding by changes in water concentration.

Echocardiographic analysis. Cardiac and valvular anatomy and function were retroactively analyzed from the patients' clinical echocardiograms when available. Left ventricular and atrial parameters were analyzed using TTEs (taken, on average, 70 days before transplant). The LV volumes and ejection fraction were measured using the modified Simpson's method (10). The LV dimensions—diameters, septal and posterior wall thickness—were measured by standard M-mode techniques (11). Left atrial dimensions were measured using validated techniques (12,13). Mitral annular diameter was measured in the four-chamber and two-chamber views (14). Two-dimensional TEEs (taken immediately before transplant) were analyzed to describe mitral valve geometry and function. In the long-axis view, the length of the leaflets was measured from annulus to leaflet tip with the leaflets open in diastole. Leaflet thickness was measured at the leaflet tip, middle, and base in diastole in the long-axis view. The degree of MR was assessed semiquantitatively by the size of the regurgitant jet (15). The presence or absence of leaflet redundancy—presence characterized by excess leaflet tissue sagging into the atrium (14)—was evaluated in the parasternal long-axis view.

Statistical analysis. Descriptive statistics were presented as mean values and standard deviations for continuous variables and as frequencies and percentages for categorical data (SAS version 8.2, Cary, North Carolina). Groups were compared using standard *t* tests and chi-square tests. Because of the many statistical tests, two-tailed significance was accepted at $p < 0.01$. Slight differences ($p < 0.05$) were also reported. To account for multiple measurements from the same valve, mixed model repeated measures analyses were performed using SAS PROC MIXED. Many variance component structures were considered, and compound symmetry was assumed in the final models after analyzing Akaike Information Criterion and other selection criteria. Independent variable transformations were tested to improve model fit and to ensure that the relation of the variable was well calibrated with outcome.

Mixed linear models for each of the biochemical parameters investigated differences between the control subjects and patients with CHF and within the CHF diagnostic subgroups of ICM and DCM. Differences in biochemical properties were analyzed between anatomic subgroups within the valve: posterior leaflet, anterior leaflet, and chordae; center and free edge of the anterior leaflet; basal and marginal chordae; and anteriorly and posteriorly inserting chordae.

Interrelations between the echocardiographically measured parameters were analyzed using univariate correlations. To determine if the concentrations of matrix components were related to the alterations in cardiac geometry and function, the echocardiographically measured parameters were also analyzed with respect to biochemical parameters. Using repeated measures mixed linear models, univariate associations were investigated for each echocardiographic measure separately to utilize information from the maximum number of data points. Thereafter, multivariate mixed linear models were constructed to determine the most important correlates of the biochemical parameters. A criterion of $p < 0.05$ was used for retention of variables in the final multivariate models. Unadjusted and adjusted p values were provided.

RESULTS

Biochemical matrix measurements. The DNA concentrations of the valves from patients with CHF—especially with DCM—were far greater than normal (Figs. 1A and 2A). This result was most prominent in posterior leaflets ($p = 0.0025$). Likewise, anterior leaflets from patients with CHF contained more DNA per tissue dry weight than controls ($p = 0.0008$). The free edge of the anterior leaflet showed the same trend in DNA ($p < 0.0001$), as did the chordae inserting anteriorly ($p = 0.031$), but the central region of the anterior leaflet had only slightly higher DNA concentrations ($p = 0.011$). For patients with DCM, the DNA concentration for all chordae was slightly higher than normal ($p = 0.033$).

The collagen concentrations in the anterior leaflets from patients with CHF were greater than in normal anterior leaflets ($p = 0.007$). This difference was also found in the anterior leaflet free edge ($p = 0.0035$) (Figs. 1B and 2B). There were no significant differences between collagen concentrations in the posterior leaflet and chordal groups as compared with the normal control subjects.

Valves from patients with CHF had significantly higher GAG concentrations, which were again most prominent in patients with DCM (Figs. 1C and 2C). The posterior leaflets contained more GAGs per dry weight ($p = 0.021$) than normal posterior leaflets. Likewise, the chordae inserting into the posterior leaflet had significantly higher GAG concentrations for patients with DCM ($p = 0.0037$), who also had slightly higher GAG concentrations in anterior chordae ($p = 0.02$).

The increased concentrations of cells and matrix of these CHF valves were accompanied by slightly lower water concentrations than in control valves ($p < 0.046$ for chordae) (Figs. 1D and 2D). Greater reductions in water concentration, however, were found in the valves of patients with ICM ($p = 0.04$ for posterior leaflets, $p = 0.0034$ for anterior chordae, $p = 0.007$ for posterior chordae).

Overall, there were no significant differences in biochemical measures between valves from ICM and DCM patients. Both groups demonstrated very similar trends when compared with the normal control groups (Fig. 2). In general, however, the valves from patients with DCM tended to demonstrate more pronounced elevations in DNA, collagen, and GAG concentration, whereas the valves from patients with ICM tended to have the lowest water concentration.

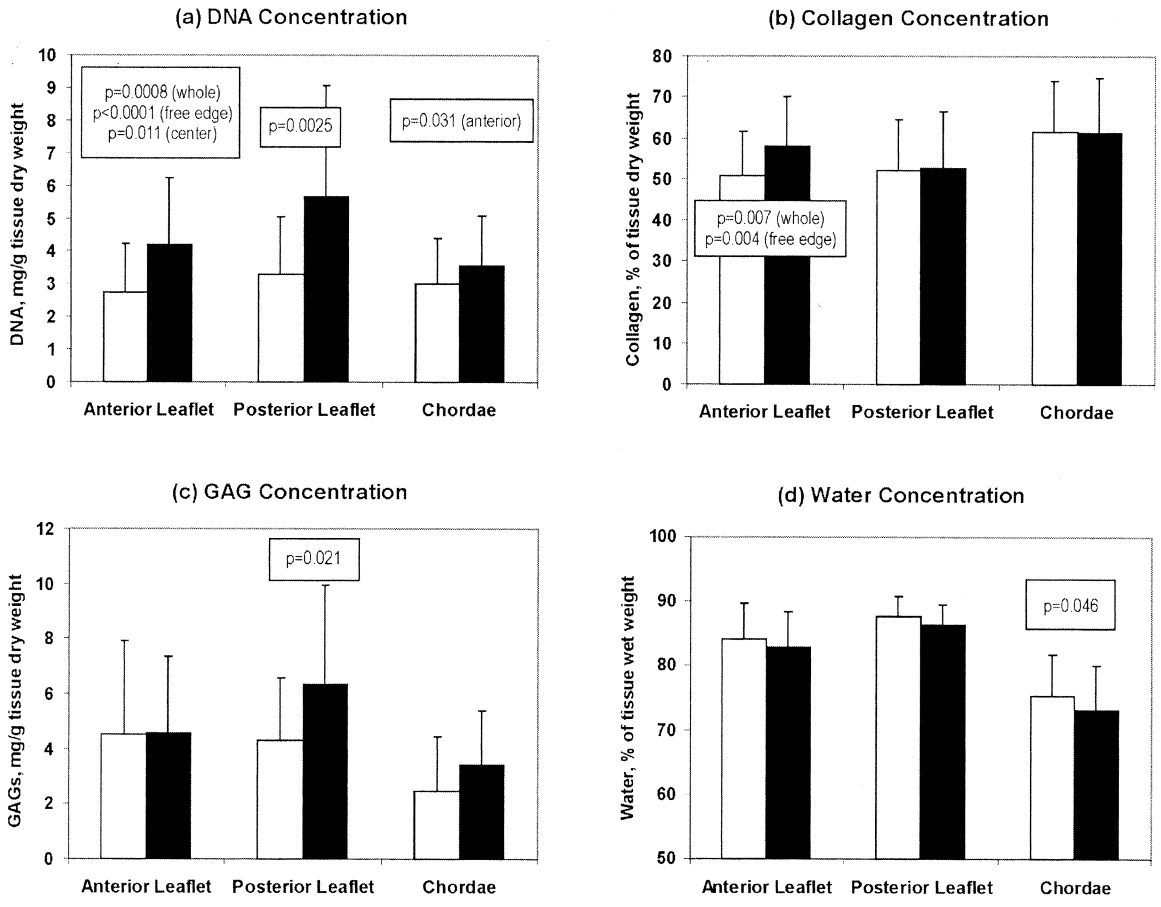

Figure 1. The extracellular matrix concentrations in control **(white bars)** and congestive heart failure (CHF) **(black bars)** mitral valves: **(a)** deoxyribonucleic acid (DNA), **(b)** collagen, **(c)** glycosaminoglycan (GAG), **(d)** water. The p values indicate difference versus control valves.

Demographic and echocardiographic measures. There were no significant differences in the mean ages of the patient group and the autopsy control group (Table 1). Gender distributions were also identical. In the primary (biochemical) analysis groups, the mean age of the DCM group was slightly less than ICM (52 ± 16 vs. 62 ± 7, $p = 0.037$), with slightly fewer male subjects (61% vs. 93%, $p = 0.034$).

The patients demonstrated the classic characteristics of heart failure (16), but also notably had moderate MR, annular dilation, and mitral leaflets that were 26% thicker ($p < 0.011$) and 28% to 41% longer ($p < 0.002$) than normal (Table 2). Leaflet length was significantly correlated with left atrial area (anterior: $r = 0.65$, $p = 0.007$; posterior: $r = 0.71$, $p = 0.014$) and annular diameter (posterior: $r = 0.7$, $p = 0.001$), whereas leaflet thickness was correlated with annular diameter (anterior: $r = 0.53$; posterior: $r = 0.66$; both $p = 0.027$), LV end-diastolic volume (anterior: $r = 0.64$, $p = 0.05$; posterior: $r = 0.82$, $p = 0.006$), and left atrial volume (anterior: $r = 0.72$, $p = 0.025$; posterior: $r = 0.70$, $p = 0.05$). The mechanism of MR in most patients was the result of annular dilation and/or leaflet restriction. The LV collagen concentrations were five times higher than normal, indicative of ventricular fibrosis. These geometric and functional characteristics were similar between patients with DCM or ICM, except for left atrial area, which was slightly higher in patients with DCM ($p = 0.03$), and MR grade, in which patients with DCM were more likely to have an MR grade of 3 or 4 ($p = 0.01$). There were no significant differences in LV dimensions between the DCM and ICM subgroups (LV internal diastolic diameter 7.5 ± 0.9 DCM group vs. 7.2 ± 0.7 ICM group, $p = 0.38$).

Statistical association analysis. There were numerous significant univariate associations between ECM concentrations and the echocardiographically measured parameters. Many of these associations, predominantly related to leaflet and annular dimensions, remained significant in the adjusted multivariate models (Table 3). The absence of anterior leaflet redundancy, in particular, was associated with almost all measures of ECM (Fig. 3). Significant positive associations were also found between anterior leaflet thickness and leaflet DNA, and mitral annular diameter and chordal collagen. Water was inversely associated with both left atrial diameter (leaflets) and LV collagen (chordae). The grade of MR, expected to be highly associated with the valve dimensions, showed a significant positive association with leaflet DNA ($p = 0.01$) and a slight positive association with chordal collagen ($p = 0.07$) in univariate models, but these associations were not significant in the multivariate analysis ($p = 0.6$ for both).

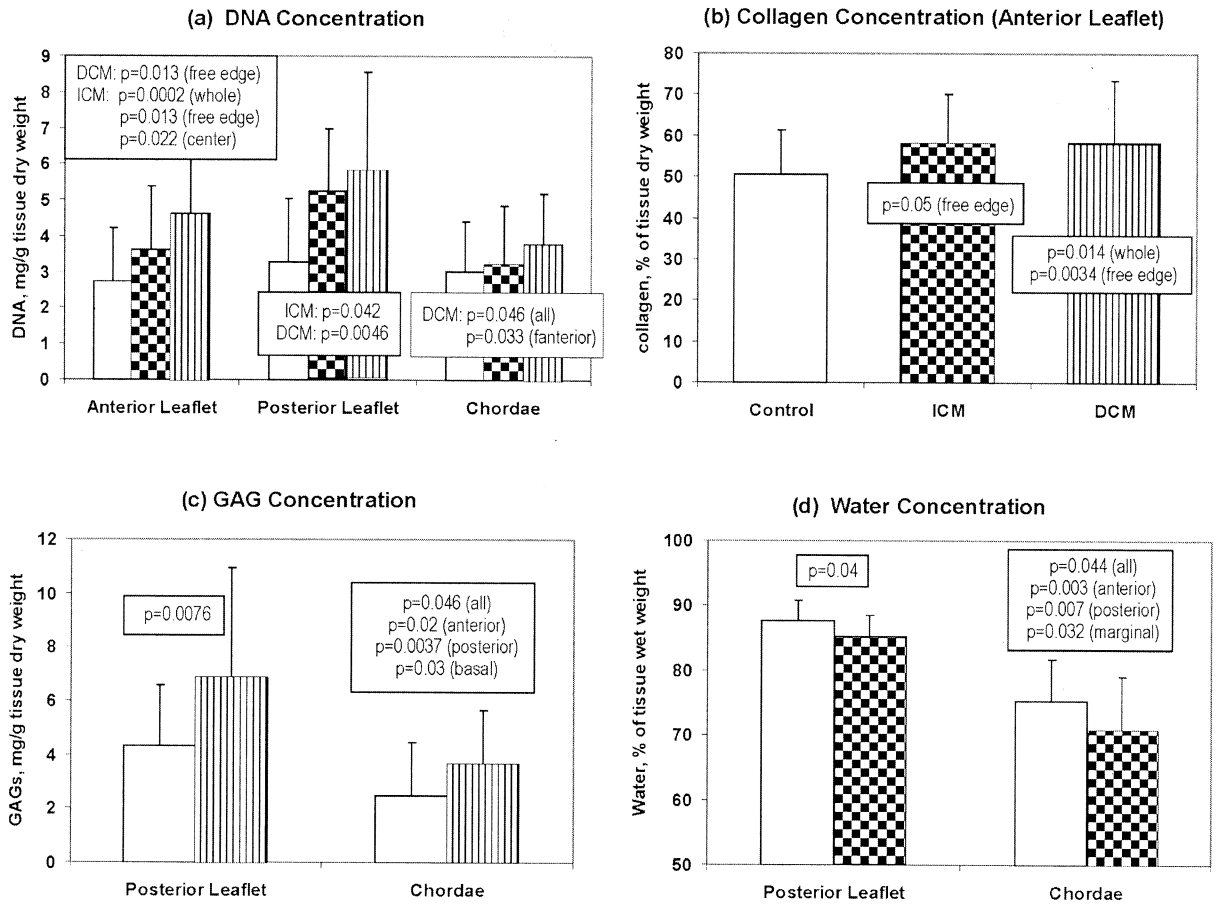

Figure 2. Significant or slight differences in extracellular matrix concentrations between control **(white bars)**, dilated cardiomyopathy (DCM) **(lined bars)**, and/or ischemic cardiomyopathy (ICM) **(checkered bars)** groups (diagnostic subgroups for congestive heart failure): **(a)** deoxyribonucleic acid (DNA), **(b)** collagen, **(c)** glycosaminoglycan (GAG), **(d)** water. The p values indicate difference versus control group.

DISCUSSION

In this study, we have demonstrated that the ECM constituents of mitral valves from patients with end-stage CHF exhibit fibrotic changes and are substantially different from those of age-matched normal control subjects. Moreover, this dysfunctional remodeling of mitral valves occurs in proportion to the significant alterations in ventricular, atrial, annular, and valvular dimensions that accompany CHF. These findings suggest that loading conditions contribute to matrix remodeling, and that the functional MR that develops secondary to ICM or DCM is associated with distinctive and abnormal structural changes in the valves.

The altered proportions of ECM, particularly collagen, in these mitral valves from patients with CHF indicate that the tissue has become heavily fibrotic. This fibrosis may represent a tissue adaptation to provide additional tensile strength (7,17), particularly in patients with CHF, who have higher than normal plasma and ventricular levels of proteolytic enzymes (18) and disruptions of the normal renin-angiotensin system (19). The excess GAGs in these tissues may also be related to fibrosis, because certain GAGs, via their association with proteoglycans, have roles in collagen fibril organization (9). The dramatically higher concentration of DNA in these valves (more cells than normal per gram of tissue dry mass) may be a result of either elevated cell proliferation or reduced cell turnover or apoptosis. These additional cells may be responsible for the altered matrix proportions, although determining how their production of ECM has been altered will require more experimental, mechanistic studies. Finally, the gains in collagen, GAGs, and cell concentrations were accompanied by a reduction in the concentration of water, consistent with the definition of fibrotic tissue as being very dense (17).

These biochemical alterations in the valve tissue provide evidence that the mitral valves have remodeled in these patients, potentially as an adaptive response to loading or geometry outside of their normal range. Our findings of elevated collagen and DNA concentrations are supported by several previous animal studies. In sheep models of ischemic heart failure, ischemia of the left ventricle and/or papillary muscles caused long-term MR and up-regulation of leaflet procollagen (collagen precursor) and alterations to the normal distribution of leaflet collagen (5,6). Likewise, increasing the mitral valve loads in a rat model of LV pressure overload caused up-regulated DNA and collagen synthesis (7).

Table 2. Valvular and Cardiac Chamber Dimensions and Functional Parameters

Measurement	Subjects	CHF	Controls	p Value (*t* Test)
AL length (cm)	19	3.22 ± 0.49	2.51 ± 0.31	0.001
AL thickness (mm)*	14	2.69 ± 0.77	2.13 ± 0.13	0.006
PL length (cm)*	18	2.31 ± 0.50	1.64 ± 0.23	0.002
PL thickness (mm)*	11	2.75 ± 0.68	2.19 ± 0.17	0.01
MV annular diameter (4C) (cm)	26	4.34 ± 0.74	3.75 ± 0.42	0.01
LVISD (cm)	32	6.77 ± 0.91	3.25 ± 0.42	< 0.001
LVIDD (cm)	32	7.38 ± 0.87	4.94 ± 0.55	< 0.001
IVS thickness (cm)	31	0.84 ± 0.20	0.98 ± 0.15	0.11
PW thickness (cm)	31	0.87 ± 0.28	0.94 ± 0.11	0.5
LA diameter (cm)	32	5.08 ± 0.89	3.74 ± 0.60	< 0.001
LA area (cm^2)†	23	27.3 ± 5.41	18.1 ± 4.22	< 0.001
LA volume (4C) (cm^3)	21	87.0 ± 28.0	46.3 ± 14.3	< 0.001
MR, graded 0–4‡	36	2.53 ± 0.99	0.43 ± 0.38	< 0.001
EF (%)	33	17 ± 6	57 ± 5	< 0.001
LVESV (cm^3)	24	253 ± 82	60 ± 13	< 0.001
LVEDV (cm^3)	24	311 ± 94	129 ± 53	< 0.001
LV collagen (mg/g dry weight)	35	51.3 ± 27.5	10.2 ± 1.0§	< 0.001

*The leaflets were inadequately imaged to determine measurements in some transesophageal echocardiograms. †The LA area in subjects with DCM was greater than in ICM (p = 0.03). ‡There were more MR grades 3 and 4 in the subjects with DCM (p = 0.01). §Control LV collagen values are from unpublished data (N.D. and C.M.).

4C = four-chamber; AL = anterior leaflet; CHF = congestive heart failure; DCM = dilated cardiomyopathy; EF = ejection fraction; ICM = ischemic cardiomyopathy; IVS = intraventricular septum; LA = left atrial; LV = left ventricular; LVEDV = left ventricular end-diastolic volume; LVESV = left ventricular end-systolic volume; LVIDD = left ventricular internal diastolic diameter; LVISD = left ventricular internal systolic diameter; MR = mitral regurgitation; MV = mitral valve; PL = posterior leaflet; PW = posterior wall.

The significant associations between the valvular ECM and the altered valvular and cardiac dimensions lend further support to our hypothesis. Many of these cardiac parameters demonstrate the dilation of the mitral annulus. In particular, the absence of anterior leaflet redundancy was a frequent predictor of remodeling (Fig. 3) consistent with stretching of the valve tissue across the enlarged annulus. Other parameters, such as MR severity, that relate to annular dilation, were associated with the cellular and matrix data in univariate models (although MR was not significant [p = 0.6] in the adjusted multivariate model).

In DCM or ICM, MR may be induced by annular dilation, abnormal ventricular wall motion and geometry, or papillary muscle displacement and subsequent reorientation of the chordae (3,4,20). Such sustained changes in the valve environment and loading, however, likely also contribute to the tissue fibrosis found in this study. We propose that the annular and subvalvular dilation causes the leaflet to become more stretched than normal across the mitral orifice, resulting in a loss of coaptation (21,22) (Fig. 4). This leaflet extension and stretching applies high tensile and membrane loads to the posterior leaflet and the free edge of the anterior leaflet, which are regions of the valve that would normally experience compressive stress relief during leaflet coaptation. Correspondingly, these regions exhibited greater magnitude changes in tissue composition than the chordae and the center of the anterior leaflet, which normally experience high tensile loads. This regionally specific remodeling is likely heavily influenced by regional changes in mechanical loading; high levels of circulating proteolytic enzymes, neurohormones, and cytokines may also induce fibrosis but would presumably affect all regions of the valve equally. In any case, the resulting fibrotic remodeling may affect the leaflet and chordal material behavior and cause organic impedance of normal valve function. It is therefore conceivable that patients with end-stage CHF have a combination of organic and functional MR (Fig. 4). However, the relative contribution of matrix change to the development of MR in patients with heart failure is difficult to determine and may be better addressed in a long-term animal model (6).

These findings of larger and thicker than normal mitral valves in patients with CHF have only been rarely discussed in published reports (23), nor were they noted in our patients' echocardiographic reports, perhaps because the valves appeared normal in proportion to the enlarged hearts. Furthermore, the altered matrix and structure in these valves raise questions regarding the surgical repair of these valves (2,24). Although surgical repair is accomplished successfully

Table 3. P Values From Adjusted Multivariate Association Models

Biochemical Measure	Cardiac Measure	Association Direction	Leaflets	Chordae
DNA	AL thickness	+	0.003	—
	AL redundancy	−	0.02	—
	LVIDD	−	—	0.05
GAGs	AL redundancy	−	0.02	0.03
Collagen	AL redundancy	−	0.002	—
	Mitral annulus (4C)	+	—	0.03
Water	AL redundancy	−	0.01	—
	LA diameter	−	0.008	—
	LVIDD	+	0.02	—
	LV collagen	−	—	0.04

4C = four-chamber; AL = anterior leaflet; GAGs = glycosaminoglycans; LA = left atrial; LV = left ventricular; LVIDD = left ventricular internal diastolic diameter.

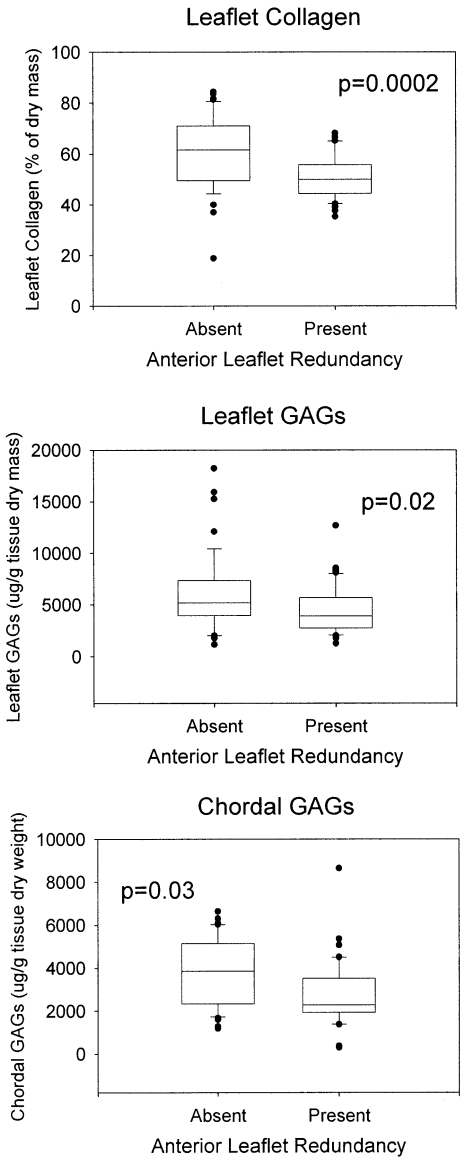

Figure 3. The extracellular matrix data segregated according to absence or presence of normal anterior leaflet redundancy. GAGs = glycosaminoglycans.

in both ICM and DCM, the long-term results are often disappointing, with recurrent regurgitation especially in patients with ICM. Although long-term failure of ischemic MR repair likely relates to an inadequate correction of the structural problem, it is also conceivable that matrix remodeling in the valve structure also contributes.

These findings of abnormalities in mitral valves from failing hearts also have widespread implications in unrelated studies of heart valve disease. Because these valves have been described as "normal" in appearance after gross or echocardiographic inspection, valves from failing hearts have served as normal or control tissues for studies of myxomatous mitral valve disease (17) and valve matrix metalloproteinases (25), and these have been utilized as a source of normal human cells for characterization of normal valvular interstitial cells (26). Our findings, however, would suggest caution

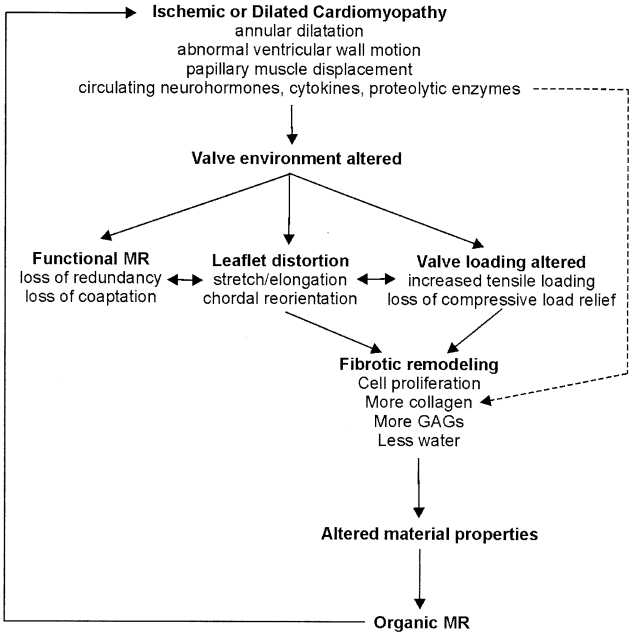

Figure 4. Proposed mechanism for secondary valvular remodeling. GAGs = glycosaminoglycans; MR = mitral regurgitation.

in the use of these valves as normal control tissue for other studies.

Limitations. There are some limitations to how data from this study may be interpreted. First, these biochemical measures cannot provide any information about how the internal collagenous matrix microstructure of the tissue, or the valvular interstitial cell phenotype, was affected. For this reason, we have incorporated histologic and immunohistochemical analyses into our ongoing investigation of this valve dysfunction. Second, leaflet thicknesses were measured using echocardiograms, which have image resolution limits, as opposed to being directly measured from the tissues. However, TEEs are the method of choice for measuring leaflet thickness in living subjects, and the TEEs from all patients and control subjects were analyzed identically. Third, more quantitative measures of MR severity, such as regurgitant jet dimensions, might have retained significant associations in the multivariate models. Fourth, it was intriguing that DCM valves demonstrated greater elevations in collagen and GAG content, whereas the ICM valves showed more reduction in water. These biochemical differences between the DCM and ICM groups, however, were not statistically significant, and both groups demonstrated the same overall trends. The finding that these two CHF etiologies do not have significant distinctions in their valvular matrix suggests that valvular remodeling is influenced less by the original etiology and more by the degree of cardiac remodeling found in these patients with end-stage CHF.

Conclusions. In conclusion, these data demonstrate that human mitral valves remodel in response to and in proportion to changes in their functional environment. Mitral valves in patients with CHF are distinctly different from

those in normal control individuals, which suggests that MR in these patients may not be purely functional.

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REFERENCES

1. Blondheim DS, Jacobs LE, Kotler MN, Costacurta GA, Parry WR. Dilated cardiomyopathy with mitral regurgitation: decreased survival despite a low frequency of left ventricular thrombus. Am Heart J 1991;122:763–71.
2. Smolens IA, Pagani FD, Bolling SF. Mitral valve repair in heart failure. Eur J Heart Fail 2000;2:365–71.
3. Levine RA, Hung J, Otsuji Y, et al. Mechanistic insights into functional mitral regurgitation. Curr Cardiol Rep 2002;4:125–9.
4. He S, Lemmon JD Jr., Weston MW, Jensen MO, Levine RA, Yoganathan AP. Mitral valve compensation for annular dilatation: in vitro study into the mechanisms of functional mitral regurgitation with an adjustable annulus model. J Heart Valve Dis 1999;8:294–302.
5. Quick DW, Kunzelman KS, Kneebone JM, Cochran RP. Collagen synthesis is upregulated in mitral valves subjected to altered stress. ASAIO J 1997;43:181–6.
6. Kunzelman KS, Quick DW, Cochran RP. Altered collagen concentration in mitral valve leaflets: biochemical and finite element analysis. Ann Thorac Surg 1998;66:S198–205.
7. Willems IE, Havenith MG, Smits JF, Daemen MJ. Structural alterations in heart valves during left ventricular pressure overload in the rat. Lab Invest 1994;71:127–33.
8. Patel J. Effect of absolute specimen size on the tensile properties of porcine aortic valve tissues. Master's thesis. Biomedical Engineering. Cleveland, OH: Case Western Reserve University, 2003.
9. Grande-Allen KJ, Griffin BP, Ratliff NB, Cosgrove DM, Vesely I. Glycosaminoglycan profiles of myxomatous mitral leaflets and chordae parallel the severity of mechanical alterations. J Am Coll Cardiol 2003;42:271–7.
10. Quinones MA, Waggoner AD, Reduto LA, et al. A new, simplified and accurate method for determining ejection fraction with two-dimensional echocardiography. Circulation 1981;64:744–53.
11. Gardin JM, Henry WL, Savage DD, Ware JH, Burn C, Borer JS. Echocardiographic measurements in normal subjects: evaluation of an adult population without clinically apparent heart disease. J Clin Ultrasound 1979;7:439–47.
12. Gutman J, Wang YS, Wahr D, Schiller NB. Normal left atrial function determined by 2-dimensional echocardiography. Am J Cardiol 1983;51:336–40.
13. Moller JE, Hillis GS, Oh JK, et al. Left atrial volume: a powerful predictor of survival after acute myocardial infarction. Circulation 2003;107:2207–12.
14. Otto CM. Textbook of Clinical Echocardiography. 2nd edition. Philadelphia, PA: W.B. Saunders, 2000.
15. Helmcke F, Nanda NC, Hsiung MC, et al. Color Doppler assessment of mitral regurgitation with orthogonal planes. Circulation 1987;75:175–83.
16. Cohn JN, Tognoni G, Glazer R, Spormann D. Baseline demographics of the Valsartan Heart Failure Trial. Val-HeFT Investigators. Eur J Heart Fail 2000;2:439–46.
17. Lis Y, Burleigh MC, Parker DJ, Child AH, Hogg J, Davies MJ. Biochemical characterization of individual normal, floppy and rheumatic human mitral valves. Biochem J 1987;244:597–603.
18. Yamazaki T, Lee JD, Shimizu H, Uzui H, Ueda T. Circulating matrix metalloproteinase-2 is elevated in patients with congestive heart failure. Eur J Heart Fail 2004;6:41–5.
19. Sun Y, Weber KT. RAS and connective tissue in the heart. Int J Biochem Cell Biol 2003;35:919–31.
20. Tibayan FA, Lai DT, Timek TA, et al. Alterations in left ventricular curvature and principal strains in dilated cardiomyopathy with functional mitral regurgitation. J Heart Valve Dis 2003;12:292–9.
21. Cochran RP, Kunzelman KS. Effect of papillary muscle position on mitral valve function: relationship to homografts. Ann Thorac Surg 1998;66:S155–61.
22. Kunzelman KS, Reimink MS, Cochran RP. Annular dilatation increases stress in the mitral valve and delays coaptation: a finite element computer model. Cardiovasc Surg 1997;5:427–34.
23. Mautner SL, Klues HG, Mautner GC, Proschan MA, Roberts WC, Maron BJ. Comparison of mitral valve dimensions in adults with valvular aortic stenosis, pure aortic regurgitation and hypertrophic cardiomyopathy. Am J Cardiol 1993;71:949–53.
24. Bolling SF. Mitral valve reconstruction in the patient with heart failure. Heart Fail Rev 2001;6:177–85.
25. Dreger SA, Taylor PM, Allen SP, Yacoub MH. Profile and localization of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) in human heart valves. J Heart Valve Dis 2002;11:875–80.
26. Taylor P, Allen S, Yacoub M. Phenotypic and functional characterization of interstitial cells from human heart valves, pericardium and skin. J Heart Valve Dis 2000;9:150–8.